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54) Title: ALPHAVIRUS VECTORS  57) Abstract  A modified alphavirus expression vector is provided with the provided with the provided alphavirus, which is a provided with the			

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### TITLE OF INVENTION ALPHAVIRUS VECTORS

#### FIELD OF INVENTION

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The present invention relates to the field of DNA vaccines and is particularly concerned with modified alpha virus vectors for use in such vaccines.

#### BACKGROUND OF THE INVENTION

10 Semliki Forest virus (SFV) is a member of the Alphavirus genus in the Togaviridae family. virus particle contains a single copy of a ssRNA genome with a positive polarity that is 5'-capped and 3'polyadenylated. It functions as an mRNA and naked RNA 15 can start an infection when introduced into cells. infection/transfection, the 5' two-thirds of the genome is translated into a polyprotein that is processed into the four nonstructural proteins (nsPl to 4) by self cleavage. Once the ns proteins have been synthesized 20 they are responsible for replicating the plus-strand (42S) genome into full-length minus strands (ref. 14). These minus-strands then serve as templates for the synthesis of new plus-strand (42S) genomes and the 26S subgenomic mRNA (ref. 1 - Throughout this application, various references are cited in parentheses to describe more fully the state of the art to which this invention Full bibliographic information for each citation is found at the end of the specification. disclosures of these references are hereby incorporated 30 by reference into the present disclosure). subgenomic mRNA, which is colinear with the last onethird of the genome, encodes the SFV structural

proteins. In 1991 Liljestrom and Garoff (ref. 2) designed a series of expression vectors based on the SFV CDNA replicon. These vectors had the virus structural protein genes deleted to make the way for heterologous inserts, but preserved the nonstructural coding region for production of the nsPl to 4 replicase complex. Short 5' and 3' sequence elements required for RNA replication were also preserved. A polylinker site was inserted downstream from the 26S promoter followed by translation stop sites in all three frames. An SpeI site was inserted just after the 3' end of the SFV CDNA for linearization of the plasmid for use in vitro transcription reactions.

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Injection of SFV RNA encoding a heterologous protein have been shown to result in the expression of the foreign protein and the induction of antibody in a number of studies (refs. 3,4). The use of SFV RNA inoculation to express foreign proteins for the purpose of immunization would have several of the advantages associated with plasmid DNA immunization. For example, SFV RNA encoding a viral antigen may be introduced in the presence of antibody to that virus without a loss in potency due to neutralization by antibodies to the Also, because the protein is expressed in vivo the protein should have the same conformation as the protein expressed by the virus itself. concerns about conformational changes which could occur during protein purification leading to a immunogenicity, protective epitopes and possibly immunopotentiation, could be avoided by plasmid DNA immunization.

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In WO95/27044, the disclosure of which is incorporated herein by reference, there is described the use of alphavirus cDNA vectors based on cDNA complementary to the alphavirus RNA sequence. Once transcribed from the cDNA under transceptional control of a heterologous promoter, the alphavirus RNA is able to self-replicate by means of its own replicase and thereby amplify the copy number of the transcribed recombinant RNA molecules.

#### 10 SUMMARY OF THE INVENTION

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The present invention is concerned with modifications to the alphavirus cDNA vectors described in the aforementioned WO 95/27044 to permit enhanced replication of the alphavirus. In the present invention, a heterologous splice site is introduced into the alphavirus replicon sequence, particularly that of Semliki Forest virus (SFV).

Accordingly, in one aspect, the present invention provides an expression vector comprising a DNA molecule complementary to at least part of an alphavirus RNA genome, which DNA molecule comprises the complement of the complete alphavirus RNA genome regions which are essential for replication of the said alphavirus RNA, and further comprises a heterologous DNA sequence capable of expression in a suitable host, such as a human or animal host, said heterologous DNA sequence being inserted into a region of the DNA molecule which is non-essential to replication thereof, and the DNA molecule being placed under transcriptional control of a promoter sequence functional in said animal or human host, wherein at least one heterologous splice site is

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provided in the DNA molecule to prevent aberrant RNA splicing of the alphavirus.

The alphavirus molecule is a large molecule and, accordingly, there is a high probability of cryptic splice sites, thereby impairing the replication of the alphavirus and hence its ability to express heterlogous DNA is impaired. By introducing the at optimal heterologous least one splice accordance with the present invention into alphavious replicon sequence, any splicing is likely to be directed at the heterologous splice site rather than any cryptic splice sites, restores the function of the SFV replicon when removed, and may improve transport of RNA from the nucleus (ref. 6).

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In the constructs provided herein, the promoter is placed upstream of the 5'-end of the alphavirus sequence, such that the resultant transcript has an authentic 5'-end, which is required for the efficient replication of the alphavirus RNA replicon.

In addition, there may be provided at the 3'end of the Semliki Forest virus segment, a hepatitis delta virus ribozyme sequence to ensure proper in vivo cleavage at the 3'-end of the sequence. Any other convenient sequence may be employed to achieve this effect.

The heterologous splice site sequence may be provided by the nucleotide sequence of the rabbit  $\beta$ -globin intron II, as described in reference 5. Such heterologous splice site sequence may be inserted into the complement sequence at any convenient location which generates perfect splice junctions. This

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precludes replication of the alphavirus, unless it is authentically removed by splicing..

I have identified five suitable sites in the SFV replicon, which are contained within an EcoRV-SpeI fragment of the replicon which is 8010 bp in length (Fig. 3). The first such site is a Ppu-MI site, at position 2719 within the EcoRV-SpeI fragment.

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In constructing the modified vectors provided herein, the EcoRV-SpeI fragment is cut with Ppu-MI at position 2719 and made blunt-ended with Mung Bean nuclease, which removes three bases from the SFV sequence. A blunt-ended  $\beta$ -globin II intron, which is 536 bp long, is ligated into the site and replaces the missing three bases with sequence added to the 3'-end of the  $\beta$ -globin intron sequence (Fig. 1).

The other four suitable sites for insertion of the Intron are the PvuII sites at bp 2518, 3113, 6498 and 6872 of the EcoRV-SpeI fragment. Insertion of the Intron is achieved by cutting with PvuII (a blunt end cutter) and the blunt-ended  $\beta$ -globin II intron sequence (Fig. 2) is ligated into one or more of these sites.

In a further aspect of the present invention, there is provided a cloning vector suitable for expression in a host cell of an heterologous DNA sequence, which comprises a DNA molecule complementing to at least part of an alphavirus RNA genome, which DNA molecule comprises the complement of the complete alphavirus RNA genome regions and has a cloning site for insertion therein of a heterologous DNA sequence capable of expression in a host cell, said cloning site being located in a region of the DNA molecule which is

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non-essential replication to thereof: promoter sequence functional in said host cell and transcriptionally controlling said DNA molecule, said promoter sequence being placed upstream of the 5'-end of the DNA molecule such that the resultant transcript. had an authentic 5' end; at least one heterologous splice set provided in the complement of the DNA molecule to generate perfect splice junctions in the alphavirus in order to prevent aberrant splicing and an additional DNA sequence at the 3'-end of the DNA molecule to direct proper in vivo cleavage at the 3'end of the reactant mRNA transcript.

#### BRIEF DESCRIPTION OF DRAWINGS

Figure 1 shows the DNA sequence of the  $\beta$ -globin intron II including three additional nucleotides at the 3'-end thereof (SEQ ID No:1);

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Figure 2 shows the DNA sequence of the  $\beta\text{-globin}$  intron II (SEQ ID No:2);

Figures 3A to 3C show the DNA sequence of the 20 EcoRV-SpeI fragment of Semliki Forest virus replicon (SEQ ID No:3);

Figures 4A to 4D show the DNA sequence of the pSFV link (SEQ ID no: 4) prepared as illustrated in Figure 5;

25 Figure 5 shows construction of pSFVlink (11060 bp) from pSFV1 using a linker sequence (SEQ ID nos: 5,6);

Figures 6A to 6D show the nucleotide sequence of plasmid pMP76 (SEQ ID no: 11, prepared as illustrated in Figures 8A to 8D;

Figure 7 illustrates subsections of plasmid pSFV link (see Figure 5);

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Figure 8A to 8D show the construction of plasmid pMP76 from plasmids pMP53, pMP70, pMP47, pMP55 and pMP71;

Figures 9A to 9B show the construction of plasmids pMP53, pMP54 and pMP55 from plasmid pMP52;

Figure 10 shows the construction of plasmid MP52 from pUC19 using a linker sequence (SEQ ID no: 7,8);

Figures 11A to 11B show the construction of plasmids pMP46, pMP47 and pMP70 from pUC19 and fragment from pSFV link, prepared as seen in Figure 7; and

Figures 12A to 12B show the construction of plasmid pMP71 from plasmid pCMV3.

#### GENERAL DESCRIPTION OF INVENTION

As discussed above, the present invention provides a modified alphavirus DNA. The alphavirus preferably is Semliki Forest virus. In particular, the present invention provides a cloning vector for heterologous gene expression in a host, such as an animal or human.

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The promoter sequence may comprise a promoter of eukaryotic or prokaryotic origin. Suitable promoters are the cytomegalovirus immediate early promoter (pCMV), although other promoters, such as the Rous sarcoma virus long-terminal repeat promoter (pRSV), since, in the case of these and similar promoters, transcription is performed by the DNA-dependent RNA polymerase of the host cell. Additionally, the SP6, T3 or T7 promoters can be used, provided that the cell has first been transformed with genes encoding SP6, T3 or T7 RNA polymerase molecules which are either inserted into the chromosome or remain episomal. Expression of

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these (SP6, T3, T7) RNA polymerase-encoding genes is dependent on the host cell DNA-dependent RNA polymerase.

The heterologous DNA insert may comprise the coding sequence for a desired product, which may be a biologically active protein or polypeptide, for example, the heterologous DNA insert may code for HIV sequences, e.g., an immunogenic or antigenic protein or polypeptide, or a therapeutically active protein or polypeptide. The heterologous DNA may also comprise additional sequences, such as a sequence complementary to an RNA sequence which is a self-cleaving ribozyme sequence.

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The DNA vectors provided herein may be administered to a host, including a human host, for in vivo expression of the heterologous DNA sequence, in accordance with a further aspect of the invention, in order to generate an immune response in the host, which may be a protective immune response. The DNA vectors may be further formulated into immunogenic compositions for such administration.

#### BIOLOGICAL DEPOSITS

Certain vectors that contain the Semliki Forest
virus replicon and referred to herein have been deposited with the American Type Culture Collection (ATCC) located at 10801 University Boulevard, Manassas,
VA 20110-2209, U.S.A., pursuant to the Budapest Treaty and prior to the filing of this application.

30 Samples of the deposited plasmids will become available to the public upon grant of a patent based

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upon this United States patent application and all restrictions on access to the deposits will be removed at that time. Non-viable deposits will be replaced. The invention described and claimed herein is not to be limited in scope by plasmids deposited, since the deposited embodiment is intended only as an illustration of the invention.

#### Deposit Summary

<u>Plasmid</u> <u>ATCC Designation</u> <u>Date Deposited</u>

10 pMP76

#### **EXAMPLES**

above disclosure generally describes A more complete understanding can present invention. be obtained by reference to the following specific These Examples are described solely for purposes of illustration and are not intended to limit the scope of the invention. Changes in form and substitution of equivalents are contemplated circumstances may suggest or render expedient. Although specific terms have been employed herein, such terms are intended in a descriptive sense and not for purposes of limitations.

Methods of molecular genetics, protein biochemistry and immunology used but not explicitly described in this disclosure and these Examples are amply reported in the scientific literature and are well within the ability of those skilled in the art.

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#### EXAMPLE 1

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. This Example describes the construction of plasmid pMP76 as outlined in Figures 5, 7, 8A, 8B, 8C, 8D, 9A, 9B, 10, 11A, 11B, 12A and 12B.

Plasmid pSFV link was created by restricting plasmid pSFV1 (Gibco) with BamHI. This plasmid was then ligated with a linker (SEQ ID no: 5 and 6) to produce plasmid pSFV link (Figures 4A to 4D, Figure 5).

Some of the SFV replicon fragments were subcloned by restricting pSFVlink with EcoRV and SpeI and 10 isolating the 890bp EcoRV-SpeI fragment. This fragment was then restricted with EcoRI and the 1906bp EcoRV-EcoRI, the 1578bp and 3627bp EcoRI-EcoRI and the 899bp EcoRI-SpeI fragments isolated (Fig.7).

The 1909bp EcoRV-EcoRI SFV fragment was cloned into EcoRV-EcoRI restricted plasmid pMP52 to produce plasmid pMP53 (Fig.9A). The 899bp EcoRI-SpeI SFV fragment was cloned into EcoRI-SpeI restricted pMP52 to produce pMP54 (Fig.9A). Plasmid pMP54 was then restricted with SpeI and made blunt-ended with Mung Bean nuclease. The plasmid was then restricted with BglII, dephosphorylated and ligated to the hepatitis delta virus ribozyme linker (SEQ ID nos. 9 and 10), that had been phosphorylated, to produce pMP55 (Fig. 9B).

Plasmid pMP52 was created by ligating a linker (SEQ ID nos:7,8), into the EcoRI site of pUC19 (Fig.10).

The 1578bp EcoRI-SFV fragment ws cloned into 30 the EcoRI site of pUC19, to produce pMP46 (Fig.11A). This plasmid was then restricted with PpuM1 and made

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blunt-ended with Mung Bean nuclease. The rabbit ß-globin intron II PCR fragment (Fig.1) was made blunt-ended with Mung Bean nuclease, phosphorylated and ligated to the PpuMI restricted pMP46 to produce plasmid pMP70 (Fig.11B).

The 3627bp EcoRI SFV fragment was cloned into the EcoRI site of pUC19 to produce pMP47 (Fig.11A).

Plasmid pCMV3, which contains the CMV promoter, Intron A sequence, BGH poly A sequence and SU40 poly A sequence, was restricted with NdeI and EcoRV. The 3191bp NdeI-EcoRV fragment was isolated and dephosphorylated. The 1321bp NdeI-EcoRV fragment was isolated and restricted with SacI. The NdeI-SacI fragment of 334bp was isolated (Fig.12A). The isolated SacI-EcoRV PCR fragment containing the 5'-end of SFV was ligated to the previously isolated 334bp NdeI-SacI fragment and the 3191bp NdeI-EcoRV fragment to produce pMP71 (Fig.12A and 12B).

Plasmid pMP53 was then restricted with EcoRI
and BamHI and ligated to the isolated and
dephosphorylated 2151bp EcoRI fragment from pMP70
(Fig.8A). This ligation was then restricted with EcoRV
and the 4057bp EcoRV-EcoRI fragment purified(Fig.8A).

Plasmid pMP47 was restricted with EcoRI and
the 3627bp EcoRI fragment isolated and dephosphorylated
(Fig.8B). Plasmid pMP55 was then restricted with
BglII, dephosphorylated and restricted with EcoRI. The
985bp EcoRI-BglII fragment was isolated and ligated to
the previously isolated EcoRI fragment from pMP47
(Fig.8B). The ligation reaction was then

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phosphorylated and the 4612bp EcoRI-BglII fragment isolated.

Plasmid pMP71 was restricted with EcoRV and BamHI then dephosphorylated. This fragment was used in a 3-way ligation with the previously isolated 4612bp EcoRI-BglII fragment from pMP47 and pMP55, and the 4057bp EcoRV-EcoRI fragment from pMP53 and pMP70, to produce pMP76 (Figs.8B and 8C).

The 5' end of the SFV replicon was produced by PCR

amplification of pSFV1 using primers SFV-5'-3 having
the sequence

5'-ATCTATGAGCTCGTTTAGTGAACCGTATGGCGGATGTGTGACATACA-3' and EcoR-SPE having the sequence

5'-TCCACCTCCAAGGATATCCAAGATGAGTGTG-3' (SEQ ID no: 9 and SEQ ID no: 10 respectively) between the CMV promoter and the 5' end of the SFV replicon. The resulting PCR fragment was restricted with SacI and EcoRV (Fig. 13; SEQ ID no: 11) and the fragment isolated.

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#### SUMMARY OF DISCLOSURE

In summary of this disclosure, the present invention provides a modified alphavirus-based expression vector wherein at least one optimal splice site is introduced to the alphavirus replicon to prevent aberrant splicing of the alphavirus genome; and improve transport of RNA out of the nucleus.

Modifications are possible within the scope of the invention.

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#### 13

#### REFERENCES

Fulginiti, V.A., Eller, J.J., Sieber, O.F.,
 Joyner, J.W., Minamitani, M. and Meiklejohn, G.,
 (1969) Am. J. Epidemiol. 89 (4), 435-448.

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- Chin, J., Magoffin, R.L., Shearer, L.A., Schieble,
   J.H. and Lennette, E.H. (1969) Am. J. Epidemiol.
   89 (4), 449-463.
- 10 3. Jensen, K.E., Peeler, B.E. and Dulworth, W.G.
  (1962) J. Immunol. 89, 216-226.
  - Murphy, B.R., Prince, G.A., Collins, P.L., Van
     Wyke-Coelingh, K., Olmstead, R.A., Spriggs, M.K.,
- 15 Parrott, R.H., Kim, H.-Y., Brandt, C.D. and Chanock, R.N. (1988) Vir. Res. 11, 1-15.
- 5. Chapman, B.S.; Thayer, R.M.; Vincent, K.A. and Haigwood, N.L., Nucl. Acids. Res. 1991, 19: 3979-20 3986.
  - 6. Huang, Zhi-ming and Yen, T. S. Benedict, Molecular and Cell Biology, July 1995, p.3864-3869.

#### 14 CLAIMS

- 1. An expression vector, comprising a DNA molecule complementary to at least part of an alphavirus RNA
- 5 genome, which DNA molecule comprises the complement of the complete alphavirus RNA genome regions which are essential for replication of the said alphavirus RNA and further comprises a heterologous DNA seuence capable of expression in a host, said heterologous DNA
- 10 sequence being inserted into a region of the DNA molecule which is non-essential to replication thereof, and the DNA molecule being placed under transcriptional control of a promoter sequence functional in said host, wherein at least one heterologous splice site is
- 15 provided in the DNA molecule to prevent aberrant RNA splicing of the alphavirus.
  - 2. The vector of claim 1 wherein said promoter is placed upstream of the 5'-end of the DNA molecule such that the resultant transcript has an authentic 5'-end.
- 20 3. The vector of claim 2 wherein said promoter is the cytomegalovirus immediate early promoter.
  - 4. The vector of claim 1 which further comprises an additional DNA sequence at the 3'-end of the DNA molecule to direct proper in vivo cleavage at the 3'-
- 25 end of the DNA molecule.
  - 5. The vector of claim 4 wherein said additional DNA sequence comprises a hepatitis delta ribozyme sequence.
  - 6. The vector of claim 1 wherein the heterologous splice site sequence is provided by the DNA sequence of the rabbit ß-globin intron II.
  - 7. The vector of claim 6 wherein the heterologous splice site sequence is inserted into the DNA molecule

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at a location which generates perfect splice junctions and restores the function of the SFV replicon when removed.

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- 8. The vector of claim 1 wherein the alphavirus is a Simliki Forest virus.
- 9. A cloning vector suitable for expression in a host cell of an heterologous DNA sequence, which comprises:

a DNA molecule complementing to at least part of an alphavirus RNA genome, which DNA molecule comprises the complement of the complete alphavirus RNA genome regions and has a cloning site for insertion therein of a heterologous DNA sequence capable of expression in a host cell, said cloning site being located in a region of the DNA molecule which is non-essential to replication thereof;

a promoter sequence functional in said host cell and transcriptionally controlling said DNA molecule, said promoter sequence being placed upstream of the 5'-end of the DNA molecule such that the resultant transcript had an authentic 5' end;

at least one heterologous splice set provided in the complement of the DNA molecule to permit aberrant RNA splicing of one to generate perfect splice junctions in the alphavirus; and

25 an additional DNA sequence at the 3'-end of the DNA molecule to direct proper *in vivo* cleavage at the 3'-end of the reactant RNA molecule.

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10. The cloning vector of claim 9 wherein said heterologous splice set is provided by the DNA sequence30 of the rabbit ß-globin intron II.

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- 11. The cloning vector of claim 9 wherein said additional sequence comprises a hepatitis delta ribozyme sequence.
- 12. The cloning vector of claim 8 wherein the alphavirus is a Semliki Forest virus.
- 13. The cloning vector of claim 8 which has the identifying characteristics of plasmid pMP76 shown in Figure 8D.
- 14. The cloning vector of claim 8 having SEQ ID no:
- 10 11.

SFV bases <u>-</u> ٣ the with the \(\beta\)-globin intron II Nucleotide Sequence of

799 750 750 750 360 480 540 120 240 300 180 atggttacaa tttgtaacga tggaaatatt ccctctgct gtatcaccat attgtctcct atcacttttt aacaattgtt catgttatat ctggctggcg ttagcttgca actttctcta agttttagag cttctcttt ccaaaccggg gatgtccctt gttgacaacc ttgtaaaatt tactctgagt atcatcctgc acttcagcac catataaatt ttttcgcta agaatgggaa tcgttaaact gattgtaagt tttctactct caggtc aatatttctg gaggataaaa catcctggtc ctttttccta ttgttcttc tttctttcac tgtaactttt ttatttgtca attatattgt ggtgttgtt gaaacaacta atcagggtat gataaggtag atgccttctt tttcattttc tgtttgagat ggacccttga aagttttcag gataattttg tcacttttgt aaccatgttc gtgagtttgg ggaggggca ggaccctcat atttttaaat tttcaaggca ataattaaat cttattggta tgatatacac cttattttct

Nucleotide Sequence of the  $\beta$ -globin intron II

420 240 300 360 120 180 480 atggttacaa tttgtaacga atcacttttt aacaattgtt tggaaatatt ccctctgct attgtctcct catgttatat gtatcaccat actttctcta ctggctggcg ctttctcttt ttagcttgca agttttagag ccaaaccggg ttgtaaaatt gatgtccctt gttgacaacc atcatcctgc tactctgagt ttttcgcta agaatgggaa tcgttaaact gattgtaagt acttcagcac catataaatt tttctactct ttatttgtca catcctggtc gaggataaaa ttgttctttc attatattgt aatatttctg ggtgttgtt tttctttcac tgtaacttt ctttttccta gaaacaacta ggacccttga aagttttcag gataattttg tttcattttc tcacttttgt atcagggtat gataaggtag tgtttgagat atgccttctt tgatatacac aaccatgttc ggaggggca ggaccctcat cttattttct atttttaaat tttcaaggca ataattaaat cttattggta gtgagtttgg

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# FIG.3A

Semliki Forest virus replicon of Eco RV-Spel Fragment

140 1080 200 260 380 1500 1020 960 099 720 780 840 006 009 540 240 300 180 gtgggcattt cgcaatccca aatacctgtt gggagtcgtc aacacctcgc gaactatctg agaccttgat agtgaaggtg ggaggccgag cgtagttctg atcatgtgaa ggtagggtac tgtcaaagga tcaaatgact gggattgaat aagactcggc gttctcggta atcgctgtac caccccgttt cgacgagcag accctccgta ggcagcggcc gtgtcgtacg cgtatgccct gaccgtcatg ccatctgtga aatacaaggc ctacaggcct cggcgcagac gggtcgtgga taggaaatta acacgatgaa gctgctgctt cccagacaat agcgagagtt aggagaggtt acggtaaaac ccacagacac agttgttagt ataccatcgt aataccactg cagacgtcac atgcaccaac caaactgggc tgactgaggg acacagtcat gctggcactt ccgacctgca ggtttgacac caaagaaact gacgcacaga tgggcgaggg tcacttactt caagaggaga gacgtactac cccggcctgt gtgtgcaaga gtcccctcaa cgaaacacta aaaccagaca agcctatggt aagaagacca cccatcgcgc gcaggtgcag ctactgagga tgtaggtgcg ggaaaaatca tgcctgcata tatgctgtac tattggattg acctacgcca gcagcatcct aaaccttgcg tctacgcaca gatagctacg agagtatcac accetegte acagccgaac catgtacaag gettttggee ggatgctgaa atcetttace cactatgtgc gggattccta atgcacctac cacaccggag aagaacacag atttagcaag ccgagagagg cgtcatcccg gagaatgatg cgaaaggctc agagatcgca ccaggacgtg cgcgtatcca aggactgtgt gaagcaattg gagcagaaag tcctaccttt cagaacggcg aagccttacc cgaccgacgt ttgtgaacgg ctctgggtgt agatgcacac ttaactcgtt ttgaagaact aagtcaccgc tggccgtata ttctccgcaa tgtacactga aaggtaaaca ttaagaaat atcacgcgga cattccctgt tggccgtcgc gcattaagat cgtcagccag cgccttccag cagaagaccc tgctggatag acgctgaatc tgaaaggtgt cgctagcagg ccaggaacat gtcagatcac ctcgacgcgt gcgcgttga ggcatactag cttccgattg gatgaaaac ccttcagagt gacgtcgacg gggtacgtag gccgtgacgt gaaagagtct cagaggatag aaacgagga ctgactagag atcggcagtg catcaggcga atgtttgacg gtgttacagg aaactgtcca ggatctacat ttccacctga tccgggaagg gctacgccag atgcgcagcg gcagccgaag

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### F1G.3

3000 3060 2760 2820 2880 2940 2160 2340 2400 2520 2580 2640 2700 2100 2460 1920 2040 1980 1620 1680 1740 1800 1860 gtactatgga cctcacccgc ggccacattg ggctgcgcct gcctgtcctg agcatttaag cgagaacaac atgtcataaa gcactacgga cacaggacag aaagcagctg ccctgcgtcg aacgctggcc cggattcttc tctggtcacc ccgcgggaag tgccgtggac cctaattgct cgcctacgaa ctttggggtt atatgacggc tttgagcgag ccatattgcc agctgaaaga agaggaagcg agcagagcag ccttgtatgc tggtgtggaa gtaactttac ttgaaggacc aaagcctggt ccataattac tttgcaccaa ccctgtatta gaggctgggc catctcaggg tcatagacac ggtgtcgtcg ctctgctggc ccaagcaatg gcactgaagt tgtctacgtt ggaaactata tccatgaatt ccaaacacga tgaagaagca tgcacctct agtttcaagc agaaagtcag gcgtcaagag tagtaggagt aggtcgacgg aatgaaaatc gaggataggc attccacagg atgaaggtga tgttgggcga gagtggagca ttgaatgaaa ccgaaggtgt acatgcttcc acagcagcag cacaacatct acggccatcg aaacccataa ctgctaaacg cattccggta tgcggagacc aagactacag agcctcgtga gttaacgacg ttggcccccg ccggtccctg tregtcaaca gagaactacg aaaaatgct aacccccgt ggcggttacc cgacaaaata gttttctgcc gcgtccagtc catcgtgtta cgaagtcatg gcagaaggtg gacgcgcact cctatcaaac agcgaacgtg gacagcagag agtggtggcc cccgtgcaac tgactccatc agtggtgtta gaacttcaac ccaggaaata ttcgcttgc cgggaggcc cgacgtagat agagctaacc ggcaccatat tattattaag gagctccaag cgaaagggag caccgacgag atcggccatt gcacgaccaa ggattaaggt aagaagaaca tccagaacaa gaatcagatt cttactctcc acagtggcct tggacgaggc ctcggagcaa agcttaaggt gacgttgcac agccaggaga accgtggaca acgccgtaag atgtactgct gcaagtctgc tgttggtggg tcaggccgtc aggagaactg gggaaaacag tggtgtacaa cgtcgctgaa agtacgtgtt ccgtgctcaa taacacataa taccatgtgg ttgacctgg gagcacgtga gaagaatggc gtggacgcgt gacactgccg gaggacagag agtatatcca ggcaagatgc accaagccca cagttggact aaaggggtat ggcgatccct aatatgatgc gggacaagta atcctatatg cttgttaaac gggctgaaga ccgggatcag agcggcaaga gtgaaaataa agggtcctac agcgccacta gttcacggac actgacgccg tegggtttgg tccccgcaga

5/39 3840 4620 3720 3780 3240 3,600 3660 3900 3960 4020 4080 4140 4200 4260 320 4380 4440 4500 4560 3420 3480 3540 3180 3300 3360 3120 caaccaggct tccggtgaac cccgctgctg tgatgacgtg tcgtaagggc aaacgaacag caaccgcagg gttctccaac gagtgccgtg taagaggca tggaactgta gggagcagca ccacgctgta cgctgtctac ccatctattc aagttgggag cgcaatgaca gaacattcac agcttacgga ctcgtctgca ggcagttatc tgagtggctg gctgcagatg agctgccagg gtgctacgac ggctttgcct gcgaattggc aatccctcaa gcagagacaa agttgctcaa gcctggtggg tgcaagaggc gatccaaatg tgtgccgcta gcgtagccat gtacgaaatt ctaacgcccg accccgtcat cagcctttaa taattcctat gcagaaagtt tgttcttgct ataccaagct cctacagagt atgccgcaac cgggcaagca gcagtagggt tggtctttgt acgccatgaa tcttgatgag agtacaacct gegeegatag aggctgcagc tggcccagac gacaacatca gaagggacc tcactgagca accatctact acggctgtgg ccggacagca tactttgaag gtgccctgcc tgcggctcgt caccagatga gttaacgcag aaatggccgt aatacagaag tgtgcaccat tatggattca cagtggcata ctggacaatg aggttcgact tgtgtcgacc cccggcggca tcctccttaa acggttaaag ctggtgagtg aatgtcacag cgctgacgtg tgacatgagg gagagtgcac actgacgttg tcccaggaca aacagtcatg aaacagactg cggaagagat gctgtactcg cgaaacaatg agcggctgtg gactgaagcg gctttctgtg ctaccagcag actgctaaaa agccgttgtt tgtcaccagc ctctacgcta cacggccggg cgtggcgaag tgagtacaag ccacgtcctg tgacgccggc tggaaggatg cctgaagggg gtcaccgctg gcacaattaa aggaagccat aagccatgca cgtgcacaga tctctgccac ccgccgaagt tgttcagcgg acgccacgga cagacttggt ctgacgggtc tggcagagat acgcgctggg catcaacacc aaatcagcga gcccggattg gaaagagacc tatgcagggc gaatccacca acagacctgg gacatacctt aaatccaacc taagagggta gactgccggc atgcgctacg ccctggtggc tcacttggtt aagaaatcc gagctgacca gctattgata gcgcctaatt cgggcagtgg tccacaggag acagcaatgg tacaqtacca atatgcctat gattccgatt gacatagcca ggggatggcg acaccagtgg agagtgttgc tttgacaacg tatgccggag ctggaagcta gcagaaagaa gtcaataaag cgacgcaggg ctaagtttag acggaattca cttgggggag tacgccgata cactgggata ctdccgcacg

5400 5700 5340 5640 5940 0909 6180 4920 4980 5040 5100 5160 5220 5280 5460 5520 5580 5760 5820 5880 0009 4740 4800 4860 cccgtgtac gtgcaacgaa atacgacgca gagaaactgc cgtggagtgc acctatccgg agctgctgcc cagattcacg ggaaagaccc ccagtctcgc cagattgtac ctgcccggcg cagtgccgtc ctcggacact cgcacaactg ggagaagctg ctcgtcttcc ctcgatctac gctgcctttg tactttcgga gaaggttctc atctacgacg aggcatcgcg gaacccgatt gcgaccggtg ttgctcatct gagcgacatt ccdccaccaa cagtgttcaa atqctaaaca aaggcccgaa ttcccatgga aacacaga catcgggggc caatcgcagc taacagatga ggtaccccg cgactgtacg atattttctc atctccagtg agagtcgata gtgacatcga tggacctcga gcgcggcgga ttaggaacaa cctccgggat atactgagag taaagtgcga ggaccaccga ctgaacccgc gcatggtggt agtatgccgc gtgctagcgg atggactcgg accaaattga gaggctaata gacaggctca tacgcggttc cccgatgtag tcgtaccaga tgcttggaca taccaccagc tgggaagaat ctgcaggagg ccagggacga aggcagcaca ccaaaattgg gatgcgttgg gaggtgat agtccgcgga gacttggact ttgcagtcgt gacgtacacc gcagaccatg cttgcctccc aggactgcgt caagttaaaa gtgcagaagg cggagaatat sacctatgtg cttggttccg cataccaaca attctcaagc aacagtggcg gtcggatagt acatcatgcg actacagaac actaccacc caaagtcact aaaatccgtt aatgtacccg gcctgcccca gcacgaggtc actaggccgc gcacccatcg cacggtggtg agtgacggct agctgcatac taggtcacac tgtagatggg ttcagtggtt acgagggttt gctacccagt ccctgaaccc gaaattacco gctacccgaa atgcctgctc agaacatcac agacccacaa aacgagatgt atttacaaca aggaggagaa aaatgcagat acatgaaagc acgtaggccg tgatcgaaag tggttgacgg ttcagaacac aaatgcgaga gcccgaagag gaaagccgac actttgacga acgtcctgcg cagatgtgca tegecegeet cgaaatacca cgacggtacc atcggtcgtt ataccatgtc ctcccatagt tacttggaca aacgtcacgc ttcaagcgct ataaccactg ttgttcgcta gtcgacatga gatgcggtcc tractgctga aaagtggaga acgggagcgg tcccctaccg tacctatcca aagctccggt ccgtcaccct ggcagcggac gcagaacgga cctccaccgc ccggcgccga acgttcggcg gacttcgacg ttcccctcc ctgttcgacc gaccactcag gagccaatgg gacctggcgg actgccagcg

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# FIG.3E

7/39 7020 0969 7080 7140 7200 7260 7440 0069 1320 7380 7500 7560 7620 7680 7740 6720 6780 6840 0099 0999 6300 6360 6420 6480 6540 cgcactatta tttacggccg cggtggctcc taaatgcgct agaagaagac agcagcaaaa aaagaatgtg gtagtgtttc ctgggggcct cgcagataag gttagggtaa agccatggcc acacctctac agacaggcga gtcgtgggtc ttttgtggg cccacttaag cgaactggag attgtttgat agacccggtt tcttacaggt cgaggcagcc cggagctatg caccatagca cggcgacgac cggcatccac aacagaaaa ggaaaaacgc ccacagtaac ctcgggtggt cattgggctc gtgtttcaga agcaggacga gcttgggggc gtatcctcat gacctgttat tggatcatag acgcaaacgt caggccactc atcagcgccg aaaccaaaga ggaaaaagag aacccccata acgtgcacac actccttggc agaggtgcgc tggacttgat gcttcaagtt cggccttcat cttacctgtg tccacccagg ttttgaacat gaagatcaac gcagcaactc gaagaaaccc gaaaattgaa taggcctccc atgcggctag tgcagaaaat tggtcacttg ctgatggcgg atgggcgaaa accgcctgcc gctgaagaca ttccggacag ggctgcaaaa aaattgagag gaattctgat ttacatccct ttggccgttg cagtacctgc actggcacgc attaacactg tccgcctgtg ttacgcccta agccaggacg ttggcgaccg gcctctcact ctatcatggg gtgctggttg gatatagcaa cggcccgtcc tractcctgc cgcagcccaa acaagaagaa gtatcttcgt ttaatacaca attaattgaa cccagcagat tgacgctgtc cgtcacacag gccgctaaca tagcaagtgg tgaggtagag ggcgtttaag cgcgatcatc gactttgttt actcactgac ctccgacaag agcggagcca aggggtggat tcacctacca aaatgctgtg attcgacaaa gggcaccgca cgctatcctg cagaacgcaa aagccgaaaa aagcaagccg gaaaatgact gggacattaa atcccgggta ccdcdcccdd gacttccagg catctaggta gattggtgcg taattcaagc taaggagact aagactttga acattgcatc tcgaagatct tatccagctg gcatgtttct tggagcagag acggagtgat tgaagatcat ttttgacag agttgggtaa gtgacgaggt caatggcat ccggtggcgc gacaatgaga gaagaaagac catgaagatt cagacatgtc tegeaategg ttagggtagg ggcggtccta cgtcgtcccc aaccaaacca gtggcactaa accttggcga taggatccag ttaatgatcc tttggggaaa agcagggtac aacatcgttc aacatggagg ggattcatag cgcctgttca cgagcactga aaagtccagg agggaattag atgtcggccg ctagagacgg atgaaatcgg

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7800	792	-	
cctgcgcaat ttaattggca	tttattttgc	aaaaaaaaa	
cgcaacaaga tattgacaca	ggatttttat	aaaaaaaaa	
tgtaacaaag gggcaactca	acgaataatt	aaaaaaaaa	
ctgtataact cggaaactcg	ttaattc	aaaaaaaaa	aaaaaaaaa
ataaccataa otccqcctca	cttaca	taatatttcc	ааааааааа
gcaatggcat	aattgga	ttggt	

# FIG.4/

Nucleotide sequence of pSFVlink

### **FIG.4E**

catcccga	cctatggtct	acaggcctcg	aatcc	cagatcacgc	attaagatgc	1500
ttttggccaa	gaagaccaag	cgagagttaa	tgttc		gccagg	9
gctgaac	aggagaa	gagaggttgg	gccg	aga	gccttaccac	$\tilde{2}$
tc	tc	gcggagacgg	gagtcgtcga	cgtcgacgtt	gaagaactag	ω
tatcacg	tgcagg		acctc	cgcgttgaaa	tca	74
agccgaacga	act	ggaaattacg	tagttctgtc	ga		20
ctccaagt	cccgt	caccctctag	agagcagg	aataa	acacataacg	36
agggccg	ggtta	cgacgga	atgacggcag	tcctac	atgtgga	2
ggccattc	gtccctga	ttcaag	gagcgaga	cgccactatg	tgtacaac	8
aagggag	caacag	aaactatacc	attgccg	cac	ctg	)4
acgagg	aactacga	aaagtcagag	ctgaaagaac	acgccga	acgtgttc	001
cgtagata	atg	aga	aggaagcgtc	tttgg	<b>ygga</b>	97
gcta	ccccgtt	atgaa	ctacga	tgaagat	gccg	220
ac	actacag	gagtc	tggggttc	ggatca	gct	28
aga	cctcgtgacc	aaacacgatc	gtcacca	gcaa	gagaactgcc	34
ggaaat	aacgac	agaa	ငgggaagg	acaagtag	gaaaacagtg	40
ctccatcc	ctaaacg	g	cgtggac	ctatat	agget	46
tegettgeea	ccggtac	gctggcc	ttgctc	gttaaacc	cggagcaaag	52
ggtgttat	gag	caatgc	att	atgatgca		28
ctt	catc	ctgaag	tcataaaa	tatatccaga	cgttgcacgc	64
gtccagtcac	gccatcg	tctacgttgc	ag	caagatgcgc	acgaccaacc	2
gtgc	cataat	atagacacca	caggacagac	caagcccaag	ccaggagaca	76
O	atgcttccga		agcagctgca	gttggactac	cgtggacacg	8
cat	gcagca	tctcagggcc	tcacccgcaa	ata	gccgtaaggc	8
agaaggtgaa	tgaaaatccc	ttgtatgccc	gtcgg	gtgaa		9
cgcac	ggataggctg	gtgtggaaaa	tggcc	cgatccctgg	attaaggtcc	0

# =1G.4C

4140 4320 4380 4500 4560 3840 4080 4200 4260 4440 3240 3660 3900 4020 3360 3420 3480 3540 3600 3960 3180 3300 acaattaaaa tctgccacga gccgaagtaa ttcagcggcg gccacggacg gaagccattg gccatgcaca tgcacagaag tgcagggccg atcagcgaag ccggattgtg aagagaccct gagataagaa cataccttcc atccaaccgc ctggtggctg agagggtacc acttggttgt ctgccggctg atccaccact atcagattga agtggcctgt agacctggtg gaagaacacg tactctccag cagaacaaag gcctaatttc agcaacggac gaaaatccag accagtgggc ggcagtggcc cacaggagtg tgacaacgga cgccggagaa ggatggcgta acgcagggtc aagtttagga ggaattcaga tgggggagat cgccgataaa agtgttgcgc catagccacg ctgggataac gccgcacgcc caataaagta ggaagctaga agaaagaaaa ggacagagct tgacctggac agaatggcaa ggacgcgttc cactgccgga gagcagcaac cgctgctgtc atctattcac gttgggagaa agagagcaga acgetgtage ctgtctaccg gtgccgtgta gaactgtagg ctttgcctcg cgtctgcaag agtggctggt gctacgacct acattcacac tgcagatgct cttacggata tctccaactt cagttatcgc accgcaggct catttaagga agaacaacca ctgccaggct ccacattgga ctgcgcctgt ctgtcctgga actatggagt gtagccatcc tccctcaacc aacgcccgtg cccgtcatcc gaattggccg agagacaaaa ttcttgctgt accaagctga tacagagtta gcctttaagg tacaacctgg gccgataggt gtctttgtga gccatgaagc ttgatgagag agaagttct ggcaagcagg attcctatca agtagggttg tgcaccaagt gccgcaacag aactttacgg gaaggaccgg agcctggtgc ataattacag ctgtattacg cggctcgtac aggggaccgc actgagcagc gctgcagcaa catctactgc tgcaccatcc caacgcagct atggccgtca cggcggcatc ccagatgaat ggacaatgta ggttaaaggc ggtgagtgag tgtcacaggc tacagaagtg tccacagggt gaaggtgatt gtggagcacc gaatgaaátt gaaggtgtcc tggattcaat gtggcatacg gttcgacttg tgtcgaccac ctccttaagc ttgggcgaaa acagactgtc cagtcatgtg ctgaagcgga gaagagatag ctgacgtgac tgctaaaacc ccgttgtttc ctacgctaca cggccgggtg cggctgtggt Lggcgaagaa caccagcaa tgaaggggca agtacaagac acgtcctgct caccgctgaa acgccggcag accagcagtg cagcagagga ttctgccc gaaggatgta tttctgtgct tatcaaacat cgaacgtgtg tggtggcctt acaaaataat

5280 5340 5340 5340 5400 5460 5520 5580 5640 5700 5760 5820 5880 5940 0009 5220 0909 4980 5040 5100 5160 4680 4740 4800 4860 4920 tttgacgagc gtcctgcgac ttacaacaaa gaggagaaaa atgcagatgc atgaaagcca gtaggccgca aattacccaa ccgaagagag aagccgacgc gttgacgggt tacccgaaac cagaacacac cggtcgttac cccatagtag gacttggtga gacgggtcgc gcagagatac gcgctgggcg tcaacacctc gcccgcctta aaataccatg acggtacctt accatgtcgc gatgtgcacc atcgaaaga gctccggtgc tccaccgcgc ggcgccgaga gttcggcgac cttcgacgac cagcggacat tgcggtccag gctgctgaaa agtggagaac gggagcggac cctatccaga cttggacatg gtcaccctt gccaatggct cctggcggca ccctaccgtg agaacggatc tgccagcgat atgcctatac ttccgattca tacactacag gttcgacccg ccactcagat gctgaccaca cagtaccact tattgatatg cacaactgga cttcggaga gattgtacac ccgtgtactc gcaacgaata acgacgcata gcccggcgaa acccgattcc gaccggtgcc tgcctttgac cggacactgg agaagctgtt agtetegeaa gtgccgtccc cggtgaacga caatgacagc ctacgacgga cgtcttccac cgatctacga gcatcgcgga atgacgtgga gctcatctt aggttctcct gtaagggcta accaggetge acgaacagat agtcgatacc tcgggggcca tacccccgcc atcgcagcgt acagatgaat gcgacattct tccgggatta atttctcct ctccagtgcg actgagaggg actgtacgca gacctcgaga gcggcggagc aggaacaagc ttgctcaatg ctggtgggtc acgaaattca caagaggcaa tccaaatgtc tgccgctacg atggtggttt aagtgcgaga tatgccgcat accaccgact gacatcgact gaacccgcag cttggacaga caggctcaca cgcggttcgg cgatgtagca gtaccagata ccaccagccg gcagcacaat aaaattggat ggctaataag gcagtcgtgt agaccatgtg gactgcgttt tgcgttggcc gggtgcatat ggctgtggag ggacagcagc gcccagactg caacatcaga gccctgcctg agttaaaagc gcagaaggta tccgcggaag cttggactgg cgtacaccct tgcctcccgc ctttgaaggt taccaacata tctcaagccc cagtggcgtc tcatgcgta ctgaacccgc acgaggtcga taggccgcgc tgtacccgcc acccatcgga cggtggtgga cggatagttg gagggtttga tgacggctga ctgccccaag aatccgttag acatgaggac tgacgttgtg aaacaatgga ggtcacacca tagatggggt tacccagttt ctgcatacct gagtgcacc tgtactcgta ccaggacagt cagtggttag

### **-1**G.4E

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6180 6240	30	36	12	#8	54	50	99	72	780	840	006	960	0	80	14	20	26	32	38	44	50	56	62	$\mathbf{x}$
atgcgagaac qcctqctccq	acatcact	acccacaact	cgagatgtca	attcaagcag	agacta	acttt	catca	gaagatctag	ccagctgt	atgtttctga	agcagaga	gagtgatc	aagatcattg	u	aag	gacgaggtta	tctaggtatg	aca	ttggtgcgtt	cccgggtaat	gcgcccggcg	Ü	gaacgcaatt	gccgaaaacg
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gccaccaaga otottcaaco	ctaaacaa	ggcccgaaag	cccatggaca	cacacagagg	tacctgtgcg	cacaca	acccagg	u	acttgatc	ttcaagttcg	tgaacatc	ccttcatc	Ü	cccatat	gtttcagacc	ggacgaa	dddddcc	atcctcatag	cctgttatac	gatcatagcg	gcaaacgttt	ggccactccg	cagcgccgta	accaaagaag
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ש ט	agaatatt	tatgtga	ggttccgc	agtcact	ggagccat	ctgtgt	gatcatc	cgacaaa	ggtggatc	acctaccaac	ctttgtttat	gact	Ca	tca	๙	cgctaacagc	caagtggt	gt	tttaaga	tacacag	taattgaatt	gcccgtcctt	agc	cctgct

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7740 77800 7860 7920 7920 7920 8040 8160 8520 8520 8520 8520 8520 8700 8760 8820 8940 8940 8940
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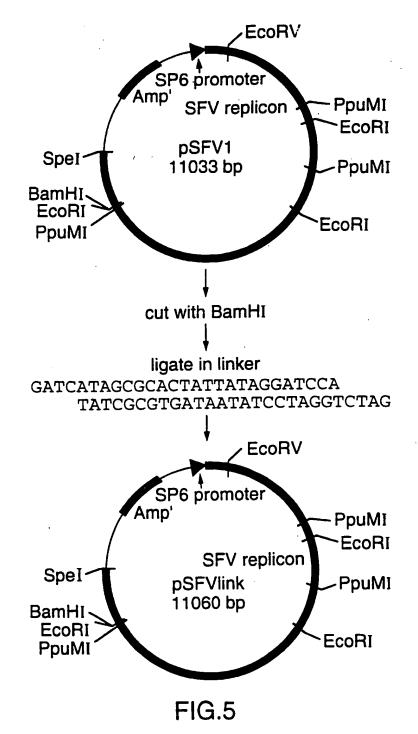
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10860 10920

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17/39 Construction of pSFVlink



SUBSTITUTE SHEET (RULE 26)

# FIG.6/

Nucleotide Sequence of pMP76

140 080 200 260 1380 540 009 720 780 900 960 420 480 099 840 360 gaccgtcatg gtgtcgtacg atcgctgtac cggttttggc ctccacccca aaatgtcgta gtctatataa caaaagattt ggccgccaaa ggcatttccg cagagcattt catcttggat cgtatgccct ggcagcggcc caccccgttt cgacgagcag cgggactttc ttatattggc acttacggta atgacgtatg tatttacggt cctattgacg atagtaatca atgcaccaac ggtttgacac ggactttcca tacacgacgc cacccacgat ctttgcagaa atgcaaatgc aagacacact aataccactg caaagaaact ccgacctgca cagacgtcac caaactgggc acggtgggag catggtgatg atttccaagt aagtccgccc catgacctta atatgtacat ctagttatta gcgttacata gacgtcaata atgggtggag ttcatcaagt gagactgaca ggaaaaatca tgcctgcata atgcccagta tcgctattac actcacgggg aaaatcaacg gtaggcgtgt atgtgtgaca agagattaac ccaaatgacc tctacgcaca gatagctacg tatgctgtac tattggattg acctacgcca atcatatgcc tgattattga atggagttcc cccgcccatt attgacgtca ctatatcata cgaaaggctc ccaggacgtg tgacagccca gcaggtcaca gatcgagcag gagaatgatg agagatcgca tcctaccttt cagaacggcg cgcgtatcca gggactttcc gtattagtca tttggcacc caaatgggcg cgtatggcgg ccgctacgcg tagcccatat catcaagtgt gcctggcatt agcggtttg tacqttgtat atgttgacat cccaacgacc acgctgaatc gtacatctac tggagtcatt cagaagaccc tggccgtata tgaaaggtgt ggccattgca tatgaccgcc cattagttca ctcgtgaccg aacgccaata cttggcagta taaatggccc tgggcgtgga tgggagtttg cccgttgacg tttagtgaac cctgccacct atattgaggc ctaccaaatt cgccttccag tgctggatag cgctagcagg gtgcatgttg tcgttcgagg tegeacetgg ataacccgc gcagagctcg tgttccagct atcggcagtg atgcgcagcg tccgggaagg gctacgccag catcaggcga tgtttgacg tcatgtccaa aatggcccgc ttcccatagt aaactgccca tcaatgacgg ctacttggca agtacaccaa ttgacgtcaa gcagccgaag attggctatt attacggggt

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## FIG.60

20/39 3600, 3660 3720 3780 3840 3900 3960 4020 4080 4140 4200 4260 3420 3480 500 3240 3540 3360 4440 30.60 3120 3180 3300 tctactctgt catcctgcct ctctgagtcc ggtcctatca acacgacaaa attgacagca aatgggaaga gttaaacttt ttgtaagtac ttcagcacag tataaattct caaagcgaac tccagtggtg aacgctggcc tttcgctatt cggattcttc cacaggacag cctcacccgc ccctgcgtcg tctggtcacc ccgcgggaag tgccgtggac cctaattgct atgtcataaa gcactacgga aaagcagctg ggataaaata tatattgtac tatttctgca ttttcctaca ggcaagaaga ccttgtatgc taactttttc atttgtcaga tcctggtcat cgttccagaa ccggaatcag gagcttactc tgaagaagca ggtgtcgtcg ctctgc tagc ccaagcaatg tcatagacac gaggctgggc catctcaggg tggtgtggaa gttctttctt tgttgtttag tcttcactt ccaaacacga gcactgaagt tgtctacgtt tcattttctg tttgagatga gccttcttct ctggacactg aatgaaaatc cagggtatat taaggtagaa aacaactaca ttggaagaat aaggaggaca agcctcgtga tgcggagacc cacaacatct acggccatcg aaacccataa acatgcttcc acagcagcag gaggataggc accettgatt gttttcaggg taattttgtt acttttgttt cctgtggacg gttaacgacg cattccggta ctgctaaacg ggtgcctgtc acctcatga tttaaattc tcaaggcaat aattaaatga tattggtaga atatacactg tacggccaca accggctgcg tacagcattt tttcgcttgc agggggcaaa tattttctt gaacttcaac gcagaaggtg gagtttgggg tattattaag ccaygaaata tgactccatc agtggtgtta gcgtccagtc cccgtgcaac catcgtgtta cgaagtcatg gacgcgcact ccatgttca gaaatattct tgattgaagg cgaaaagcct gcaccataat tgtctcctct tgtaacgaat cactttttt caattgttat ggttacaatg cctctgctaa agggtaactt atcaccatgg gacgttgcac agccaggaga accgtggaca ggattaaggt gcaagtctgc aggagaactg gggaaaacag tggacgaggc ctcggagcaa agcttaaggt gcacgaccaa acgccgtaag atgtactgct tgttatatgg gagcacgtga agcttgcatt aaaccgggcc aacattccac ataatgaagg tgtgttggg gaggagtgga cttgttaaac agtatatcca ggcaagatgc gtaaaattca tgtcccttgt tgacaaccat tttctctaat tttagagaa ggctggcgtg tctctttat ccgggatcag agcggcaaga gggacaagta aatatgatgc ggcgatccct atcctatatg accaagccca cagttggact aaaggggtat

## FIG.6[

5220 S 5280 % 5340 5580 5640 5700 5760 5820 5940 0909 5040 5160 5400 5460 5520 5880 4860 4920 4980 5100 4620 680 4740 4800 cacgactgaa agtaaacaga cggcggaaga ggacgctgac cattgacatg ggtgagagtg gtcgctgtac gatactgacg accetetacg gcacacggcc agaagcggct ggccgtggcg taaaacagtc gggcgaaaca ttgtgtcacc ggctgagtac gtaccacgic ccactaccag acgactgcta cgaagccgtt cctgttttct tggtggaagg cttcctgaag accgctttct gttgtcaccg ggctgacgcc tggccgccga tggacgccac ccacagactt atatggcaga atttctctgc gagtgttcag tccaggaagc ccactgacgg tatacgcgct ataaaatcag tgcgcccgga acggaaagag gagaagccat ccacgtgcac gcgtatgcag tgggcacaat ataacagacc ctagacatac gaaaaatcca aagtaagagg tcagaatcca gagatgcgct tggacagtgg acgccctggt gggtcacttg taggactgcc taccgggcag gtggagctga ggctacagta gctgctattg cagatatgcc gtagcgccta ctgtccacag ttcacagcaa gagaagaaaa aactttgaca ggatacgccg gcaagagtgt gtgtatgccg gcagacatag gtaggggatg gcaacaccag ggagttgacc aaccactggg aggctggaag atcgcagaaa aggctgccgc ctggtcaata cctcgacgca gacctaagtt cacacggaat atgcttgggg catccacgct ggccgctgtc catcccgctg caaccatcta caaaagttgg caatgatgac gggtcgtaag attcaaccag ggcaaacgaa gctgttctcc gctgagtgcc agttaagaga caagggagca taggtgctac gagagettae ccgtggaact ggttgagtgg cctggctttg gaagctgcag gttctcgtct caagtactat ttacgagaac aacagctgcc gcaggcagtt tatcaaccgc tgtgaacatt cagctaacgc gcagcgtagc agcaatccct actgcagaga tggagttgct gcagcctggt aaggtacgaa gactgcaaga cgtcagcctt cgtaccccgt accgcgaatt tcaatgccgc taagcagaaa aagtgttctt tgaataccaa catcctacag tgtccctgta atacgggcaa gtgagtacaa caggcgccga acttggtctt accacgccat gcatcttgat aaatttgcac atgtaattcc aaggcagtag gggtgtgcac gtggttaacg aagaaatggc tgtgcggct gcggaagggg ctgtcactga gataggctgc gtgaccatct aggacggctg cacccggaca tegtactttg tgtggccca cagtgtgtcg gttcctcct ctacaccaga gccttgaatg gcccgaagg gtgctggaca aagacggtta ctgctggtga ctgaatgtca ggcaggttcg agcaatacag atgtatggat gggcagtggc aaacccggcg

## F1G.61

22/39 7440 6780 0069 7080 7140 7200 7260 7320 7380 0969 7020 0999 6720 6840 6300 0099 6240 6360 6420 6480 6540 cacactacag agaactaccc ctccggagaa acaaaaatcc gaaaatgtac ccgcatacca aagattctca cccaacagty cgggtcggat gaaacatcat cactacctat caacttggtt gcgactaggc gatgcaccca agccacggtg gcacctgaa gacgcctgcc cgagcacgag ccttaggtca accttcagtg gtcgctaccc agtagtgacg gagagctgca acctcccagg gttacgaggg ccatgtagat ccgtgatcga acatggttga ggtgctaccc cctttcagaa cgcaaatgcg ctaagaccca agaacatgaa ccagaaatta gctatgcctg ctgagaacat cagatcggtc cgagaaagcc gcgactttga gacatttaca tccaggagga tgaaaatgca cggacgtagg cgcgcccgaa acgacgtcct acccgacggt gcgataccat tggctcccat cggcagatgt attcatcaac ggatcgcccg tcccgaaata gtcccgtcac tgcaacgtca tgcttcaagc gcatacttgg gcgaagctcc cggataacca tactccccta gaatacctat gccttgttcg attectecae actggcagcg ctgttgctgc cgcaaagtgg cacacgggag gcggacctgg gtgccggcgc ttgacgttcg ggagacttcg ctggatgcgg ctcctgttcg acggaccact tccactgcca tacgagccaa aacgattccg acagcagaac tettteece tgaatacgac acgcagtgcc caagagaaac caacgtggag acaacctatc gaaagctgct ggccagattg agcgtgcaac attctgcccg ggagcgaccg gattactttc ctcctcggac gtgcgcacaa gagggagaag ataccagtct ccgccccgtg cgactcgatc cgcaggcatc cgagaacccg caagctgcct ggtttgctca cgagaaggtt cgcatctacg cgactcgtct atgtccggtg ctacgcaatg cggcagtgtt tagcaatcgc agataacaga acagagcgac agccgactgt cggccgccac aatatgctaa tgaaaggccc tggatactga tcacatcggg ttcggtaccc acctgaacc cccdcdcddc acaatctcca ataagagtcg actggaccac atgtggacct cgtttaggaa tggcctccgg catatattt tcagatccaa gcctgtgccg aaagcatggt ggaagtatgc cgtgtgacat aggtaaagtg agccccgatg gcgtcgtacc agttgcttgg gcgtaccacc aacgtgctag accatggact tattgggaag gtggacaggc acatacgcgg gtgaccaaat cgcgcgggtg gttaggcagc ccgccaaaat teggaggeta cccgcagacc taccttgcct gtcgatgcgt atggacaaca caccaagtta ggggtgcaga gttagtccgc tttgacttgg agtttgcagt gctgacgtac ccaaggactg acagtgccct

## FIG.6

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F1G.6G

0500 10620 10020 10080 10140 10200 10260 10320 10380 10440 10560 10740 0966 9900 0996 9720 9780 9840 9300 9360 9420 9480 9540 0096 ccgaccgctg gaggcggttt aatcagggga gtaaaaaggc aaaatcgacg ttccccctgg tcagttcggt tatcgccact cttccagtc tgtccgcctt ggaacaaaac gttcccttta aagcctgggg tcgttcggct cgaaggagga gccagccatc ccactgtcct ctattctggg ggcatgctgg tgtgaaattg tcacggaaac tccaaaaaaa attgatatag taactgtata taagcttaat aaaaaaaaa taccaggcgt accggatacc tgtaggtatc cccgttcagc agacacgact gacaatagca gatactgccc tccaqctttt ctgtttcctg ataaagtgta tcactgcccg cgcgcgggga ctgcgctcgg ttatccacag gccaggaacc gagcatcaca ggtgccactc aggtgtcatt gaagcttaca ttttaatatt cctgggcatc acgctcgagt aggcaatggc catataacca gtggtccgcc aaaaaaaaa tagctcacgc gcacgaaccc ggtaatacgg ccccctgac actataaaga tgcgttgcgc aatcggccaa cctgccgctt caacccggta ggattgggaa taggtggagc atggtcatag agccggaagc cactgactcg cgcggtccga cctgaactta gaccctggaa ttgtctgagt gcagggtaag ccagcaaaag tgcaattggt aaaaaaaa taagcaatgg aattggcccc gcaataattg aagttagggt ctgttccgac cgctttctca tgggctgtgt gtcttgagtc cttcctcgct actcaaaggc gagcaaaagg ataggctccg acccgacagg tcacattaat tgcattaatg agcctagttc tggcgtaatc acaacatacg aaaaaaaa taagggagat ttgcatcgca gcaaggggga tctcgaccat agacctgcgc acattaattg tattttattt tccacctcct ctccgcagat aaagttaggg tgccttcct gtatcagctc gcgtttttcc aggtggcgaa gtgcgctctc ggaagcgtgg cgctccaagc ggtaactatc acaattccac gtgagctaac tegtgecage cgctcttccg aagaacatgt atttcgagct attggattt gcatggcatc gggcaggaca ggctctagga acgccatgcc ttgcattggg gaaaacagaa aaqcqcaaca tcatattgac aaaaaaaaa ctcggatggc ccctccccg aatgaggaaa gcgtattggg gcggcgagcg taacgcagga cgcgttgctg ctcaagtcag aagctccctc tctcccttcg gtaggtcgtt cgccttatcc ttatccgctc tgcctaatga cgcacgtcca tgttgtttgc ttcctaataa gtgagggtta gggaaacctg ttgtggtcac tegaegaata aaaaaaaa aaacgggtcg gggtggggtg ggatgcggtg catgatcctg caagaaatt acttgtaaca teggggcaac

# FIG.6F

25/39 12240 11700 11580 11940 1160 11460 11520 11640 11760 11820 11880 12000 2060 .2120 11400 11220 11280 11340 10980 11040 11100 cacctgaatc tgagtaacca tacaaacagg attccgtcag tgccatgttt cacctgattg aattaaccaa caccgaggca caacatcaat caccatgagt cttgttcaac tattcattcg tggaatttaa ttgatgagag tttattcaac ccatagttgc gaacggtctg aaaactcacg acagttacca gctgactcat ctacagagtt tctgcgctct aacaaaccac aaaaaggatc ttttaaatta tatcaggat agaggcataa gcatccatgt tcttccaga accaaaccgt aaaggacaat acaatattt atcgcagtgg acqctacctt tagattgtcg tgttacaacc ccgactcgtc agtgagaat acttggtctg tttcgttcat agaaggtgtt ggagccacgg ctttgccacg aaaagttcga aatttattca ggagaaaact gtatttggta tgatccggca acgcgcagaa cagtggaacg acctagatcc gtaggcggtg atacaatcga atcgctgtta tttcccgggg gatggtcgga atcattggca atataaatca actcgcatca cagcgcatca ctgtaatgaa gtctgcgatt aaggttatca cttatgcatt gctctgccag atgaaactgc tagaaggaca tggtagctct gcagcagatt gtctgacgct aaggatcttc atatgagtaa gatctgtcta tgcctcgtga agaaagtgag tgaacttttg tcaactcagc agcgaggtat cgggcttccc atggcaaaag ggaacactgc ggaatgctgt taaaatgctt catctgtaac atttataccc aaagccgttt cctggtatcg cgtcaaaaat catcaaaatc gaaatacgcg cgagcatcaa tcatccagcc tcagcgtaat ggattagcag acggctacac gaaaaagagt ttgtttgcaa tttctacggg gattatcaaa tctaaagtat ctatctcagc cgctgaggtc ttggtgattt atctgatcct tccggtgaga ttacgctcgt tgagcgagac aaccggcgca ggagtacgga ctgaccatct tctggcgcat tcgcgagccc aaaactcat tattttgaa atggcaagat aatttccct tctaatacct tcccgtcaag gttaccttcg agtgaggcac 9999999999 aatcgcccca ggtggaccag aagatgcgtg actggtaaca tggcctaact ggtggtttt cctttgatct ttggtcatga ttaaatcaa aatcgaatgc tgcatcatca gacgactgaa tgattgcgcc ggatattct cagaaacaac cccgacatta ttctgattag tcaatacca gttccatagg acaacctatt aggccagcca ccagtttagt ggcagcagcc atgcttaatc accaggcctg ctttgttgta cgttgtcggg aaagccgccg ctgactccgg cttgaagtgg gctgaagcca cgctggtagc tcaagaagat ttaagggatt aaaatgaagt

## F1G.61

12420 12474 12360 ttgtattact gtgcaatgta tgat ataacaccc tttttatctt cccccgagct tgatgatata tracacaca aatatggctc ttcccgttg ttattgttca caacgtggct gcagacagtt gagcaagacg ttttgagaca tegeggeete gtttatgtaa acatcagaga

8677 9544 BamHI site for insertion of heterologous inserts Hepatitis Delta virus ribozyme (antigenomic) - 9543 - 3678 Rabbit (-globin intron II 3679 -(before intron) 684 SFV replicon (after intron) 4252 12342 - 11503 682 Kanamycin Gene SFV replicon CMV promoter

27/39 Subcloning of the SFV replicon

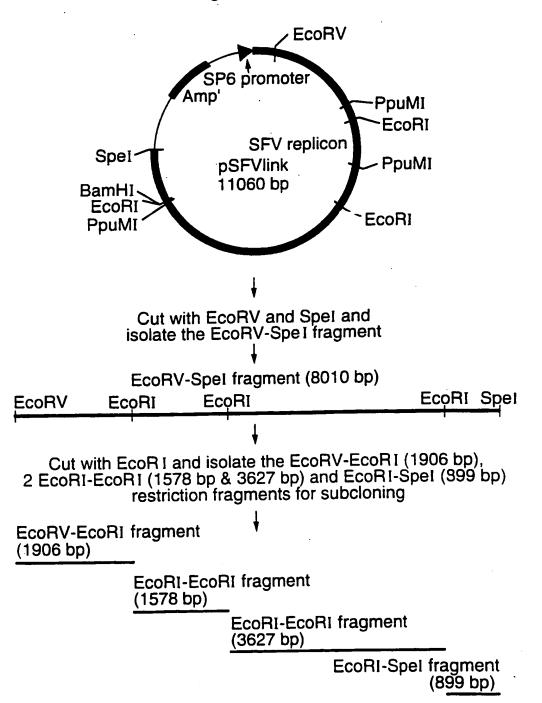
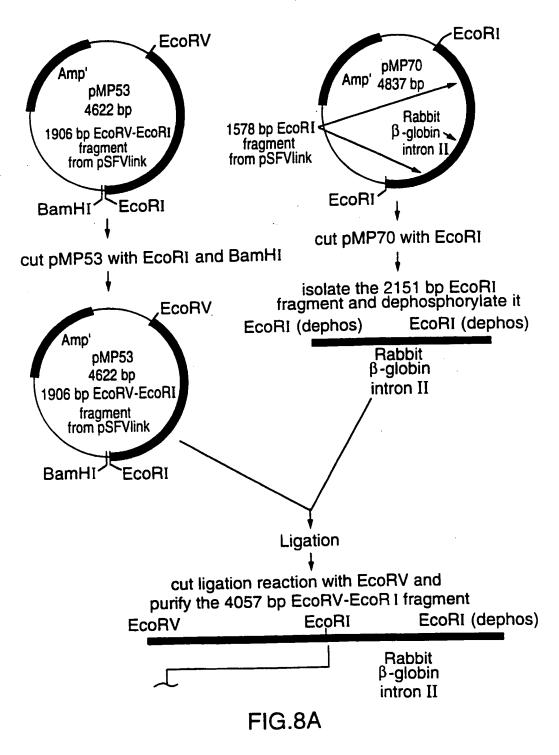


FIG.7

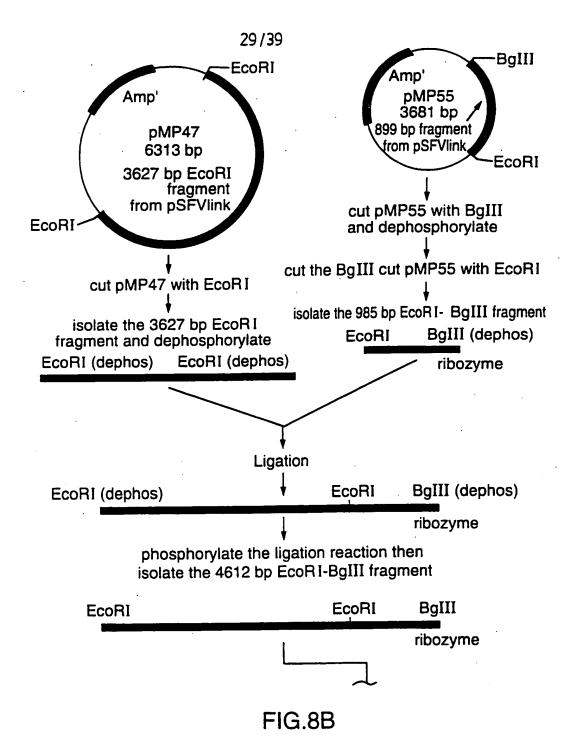
WO 99/25859 PCT/CA98/01065

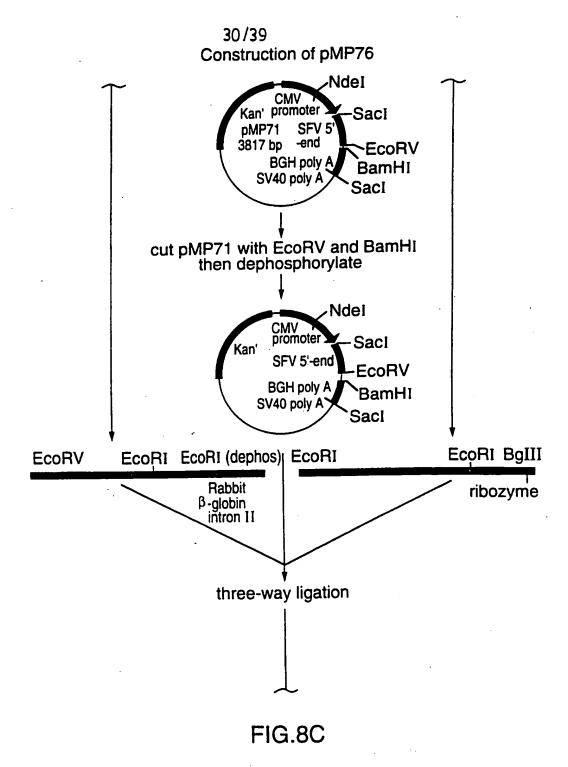
28/39 Construction of pMP76



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WO 99/25859 PCT/CA98/01065





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### Construction of pMP76 (cont'd)

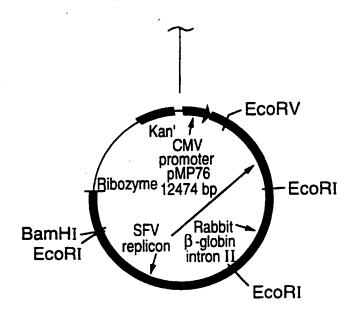


FIG.8D

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### Construction of pMP53 & pMP54

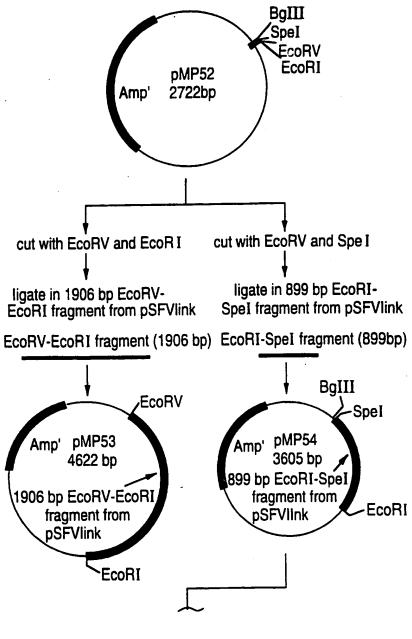


FIG.9A

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Construction of pMP55

cut pMP54 with Spe I and make blunt-ended with Mung Bean nuclease

cut with BgIII and dephosphorylate

ligate in phosphorylated linker-Hepatitis Delta virus ribozyme (antigenomic)

CGGGTCGGCATGGCATCTCCACCTCCTCGCGGTCCGACCTGGGCA...
GCCCAGCCGTACCGTAGAGGTGGAGGAGCGCCAGGCTGGACCCGT...

- ...TCCGAAGGAGGACGCACGTCCACTCGGATGGCTAAGGGAGA
- . . . AGGCTTCCTCCTGCGTGCAGGTGAGCCTACCGATTCCCTCTAG

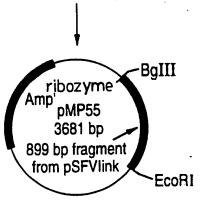
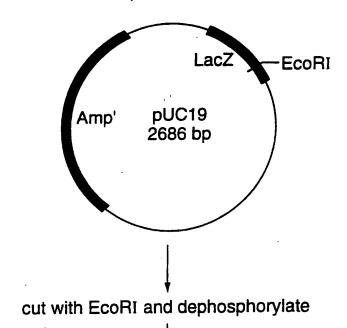


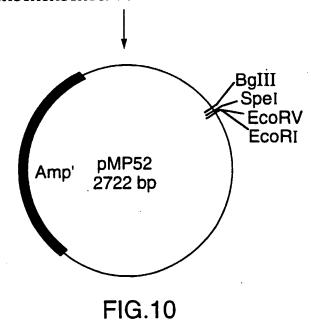
FIG.9B

34/39 Construction of pMP52



ligate in phosphorylated linker

AATTCATGATATCATACTAGTATATAGATCT GTACTATAGTATGATCATATATCTAGATTAA



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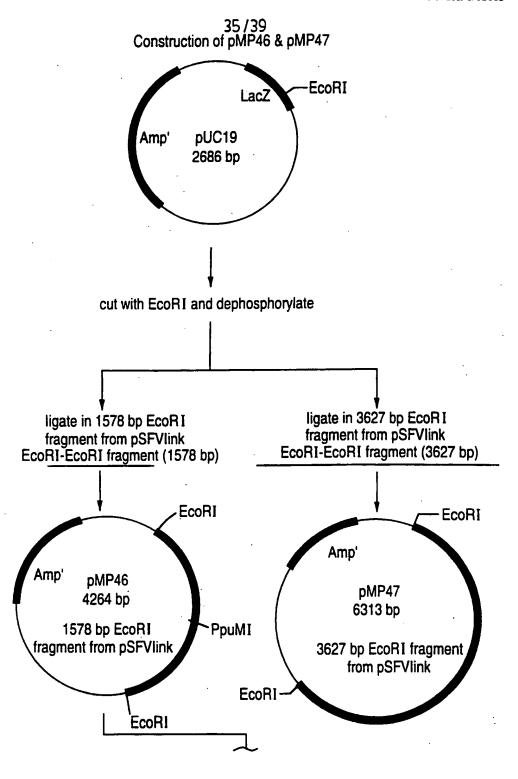


FIG.11A

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#### Construction of pMP70

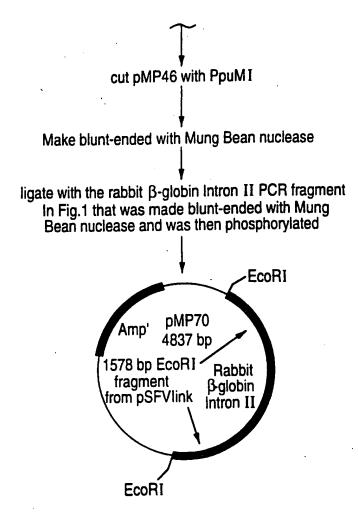
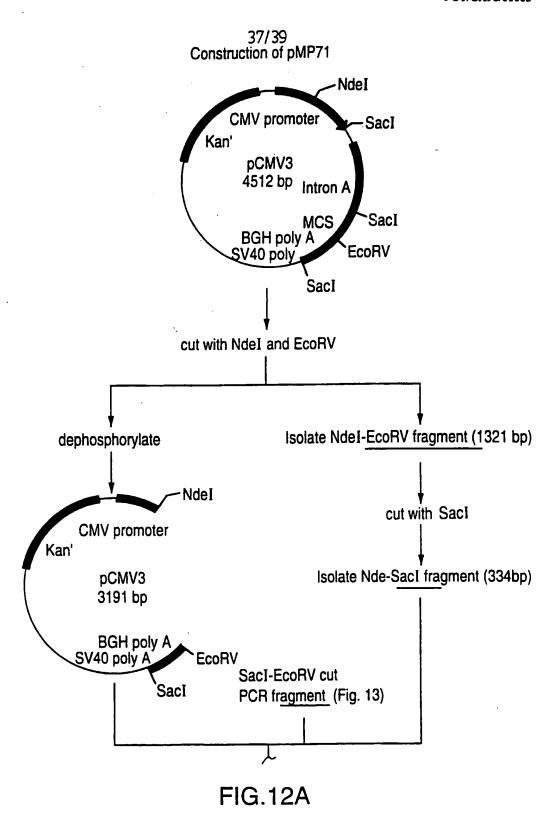


FIG.11B



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### Construction of pMP71 (cont'd)

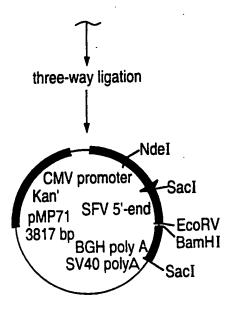


FIG.12B

# FIG. 13

<del></del> -	CGTTTAGTGA	ACCGIATEGC	CGTTTAGTGA ACCGTATGGC GGAIGIGIGA CAIACACGAC GCCAAAAAAI 30	CAIACACGAC	פררשששששום	0
51	TTTGTTCCAG CTCCTGCCAC CTCCGCTACG CGAGAGATTA ACCACCCACG 100	CTCCTGCCAC	CTCCGCTACG	CGAGAGATTA	ACCACCCACG	100
101	101 ATGGCCGCCA AAGTGCATGT TGATATTGAG GCTGACAGCC CATTCATCAA 150	AAGTGCATGT	TGATATTGAG	GCTGACAGCC	CATTCATCAA	150
151	151 GTCTTTGCAG AAGGCATTTC CGTCGTTCGA GGTGGAGTCA TTGCAGGTCA 200	AAGGCATTTC	CGTCGTTCGA	GGTGGAGTCA	TTGCAGGTCA	200
201	201 CACCAAATGA CCATGCAAAT GCCAGAGCAT TTTCGCACCT GGCTACCAAA 250	CCATGCAAAT	GCCAGAGCAT	TTTCGCACCT	GGCTACCAAA	250
251	251 TTGATCGAGC AGGAGACTGA CAAAGACACA CTCATCTTGG AT	AGGAGACTGA	CAAAGACACA	CTCATCTTGG	AT	292

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### INTERNATIONAL SEARCH REPORT

Inten anal Application No PCT/CA 98/01065

			1C1/CA 30/01003	
A. CLASS IPC 6	SIFICATION OF SUBJECT MATTER C12N15/86			
According	to international Patent Classification (IPC) or to both national classifi	cation and IPC		
	SEARCHED			
Minimum di IPC 6	ocumentation searched (classification system tollowed by classifica C12N	tion symbole)		
Documenta	ation searched other than minimum documentation to the extent that	such documents are includ	ed in the fields searched	
Electronic o	data base consulted during the international search (name of data be	sse and, where practical, s	earch teims used)	
C. DOCUM	ENTS CONSIDERED TO BE RELEVANT			
Category *	Chation of document, with indication, where appropriate, of the re	levant passages	Relevan	t to claim No.
Y	WO 95 27044 A (BIOPTION AB ;LILJI PETER (SE); GAROFF HENRIK (SE)) 12 October 1995 cited in the application		1-14	
Υ	see the whole document, especial lines 12-22 WO 96 40945 A (CONNAUGHT LAB ;LI		1-14	
,	(CA); EWASYSHYN MARY E (CA); SAME 19 December 1996 cited in the application see the whole document, especial lines 2-9; page 14, lines 15-21; 23, lines 18-23	BHARA SU)		
A	WO 96 17072 A (VIAGENE INC) 6 Jur see the whole document	e 1996	1-14	
	-	-/		
X Furth	ner documents are listed in the continuation of box C.	X Patent family me	imbers are listed in annex.	
"A" docume	regories of cited documents :  Int defining the general state of the lart which is not send to be of particular relevance.	or priority date and n cited to understand ti	ned after the international filing da of in conflict with the application b he principle or theory underlying t	ut
	ocument but published on or after the international	invention "X" document of particular	relevance; the claimed invention	
"L" document which is citation	nt which may throw doubts on priority claim(s) or s cited to establish the publication date of another	involve an inventive s "Y" document of particular cannot be considered	d novel or cannot be considered in step when the document is taken relevance; the claimed invention d to involve an inventive step whe	alone n the
other n	neans of published prior to the international filing date but		id with one or more other such do tion being obvious to a person sk the same patent family	
Date of the a	actual completion of the international search	<del> </del>	international search report	
23	3 April 1999	03/05/199	99	į
Name and m	nating address of the ISA European Patent Office, P.B. 5818 Patentiaan 2	Authorized officer		
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#### INTERNATIONAL SEARCH REPORT

Inter: Jnal Application No PCT/CA 98/01065

	· ·	PCT/CA 9	8/01065
	ALION) DOCUMENTS CONSIDERED T BE RELEVANT		
Calegory *	Citation of document, with indication, where appropriate, of the relevant passages		Relevant to claim No.
A	ZHOU X. ET AL.: "Self-replicating Semliki-Forest virus RNA as recombinant vaccine" VACCINE, vol. 12, no. 16, 1994, pages 1510-1514, XP002089524 cited in the application see the whole document		1-14
	LILJESTROEM P. ET AL.: "A NEW GENERATION OF ANIMAL CELL EXPRESSION VECTORS BASED ON THE SEMLIKI FOREST VIRUS REPLICON" BIO/TECHNOLOGY, vol. 9, December 1991, pages 1356-1361, XP000616021 cited in the application see the whole document	·	1-14
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#### INTERNATIONAL SEARCH REPORT

information on patent family members

Intern. del Application No PCT/CA 98/01065

Patent document cited in search repo	1	Publication date		Patent family member(s)	Publication date
WO 9527044	A	12-10-1995	AU	699384 B	03-12-1998
			AU	2155795 A	23-10-1995
			CA	2184261 A	12-10-1995
			EP	0753053 A	15-01-1997
			FI	963860 A	27-09-1996
			JP	9511143 T	11-11-1997
WO 9640945	A	19-12-1996	AU	695527 B	13-08-1998
•			AU	6117696 A	30-12-1996
		·	CA	2223610 A	19-12-1996
•			EP	0832253 A	01-04-1998
•			US	5843913 A	01-12-1998
			US	. 5880104 A	09-03-1999
WO 9617072	A	06-06-1996	AU	4594996 A	19-06-1996
			EP	0797679 A	01-10-1997
			US	5814482 A	29-09-1998
			US	5843723 A	01-12-1998
			US	5789245 A	04-08-1998